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Extraction of steroid diconjugates using Amberlite XAD-2 resin*

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Amberlite XAD-2 resin has been successfully applied to the extraction of steroid conjugates from biological fluids such as urine and bile. Steroid conjugates are usually almost quantitatively adsorbed on the XAD-2 resin and subsequent washing with distilled water removes most of the solids present in the biological fluids. The conjugates on the resin can be quantitatively recovered in succession on elution with methanol.

During an investigation of the biliary metabolites of [4-^{14}C] testosterone (^{14}C-T) and [7-^{3}H]testosterone 17-sulphate (^{3}H-TS) in the rat, we observed that the use of the XAD-2 resin as a means of separating steroid conjugates from rat bile resulted in relatively poor recoveries of the radioactivity in the methanol fraction, whereas the biliary metabolites of [1,2-^{3}H] testosterone 17-glucosiduronate (^{3}H-TGA) in the rat usually gave more than 90% recoveries employing the same method. In the latter study ^{3}H-TGA was metabolized predominantly to $C_{19}O_2$ steroid monoglucosiduronates. In contrast, ^{3}H-TS was biotransformed mainly into $C_{19}O_2$ and polar hydroxylated steroid ($C_{19}O_3$, $C_{19}O_4$, etc.) diconjugates (mostly as disulphates). Simultaneously administered ^{14}C-T, which was metabolized to $C_{19}O_2$ and polar hydroxylated steroid conjugates, afforded relatively poor recoveries using the XAD-2 resin method. Thus, the increased hydrophilic nature of the ^{3}H-TS and ^{14}C-T metabolites due to hydroxylation of the steroid moiety and/or to diconjugation might reduce the affinity of these conjugates for the XAD-2 resin.

In the present study, the biliary metabolites of ^{3}H-TS and ^{14}C-T in the rat were obtained by Sephadex LH-20 column chromatography. These biliary metabolites were not adsorbed completely on the XAD-2 resin and modified procedures are described for their improved recovery from the resin.

As a standard procedure for the separation of steroid conjugates, we employed the following condition: the column packed with Amberlite XAD-2 resin (100 g) was washed with 400 ml of distilled water following adsorption of the steroid conjugates and then eluted with 400 ml of methanol. Each conjugate fraction was processed by this procedure. Thus, it became apparent that the diconjugates were not adsorbed completely on the XAD-2 resin, in comparison with other conjugate fractions. In order to obtain information concerning the nature of the diconjugates, these aqueous and methanol fractions were solvolyzed, extracted with ethyl acetate and examined by

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TLC. No substantial differences were observed in their hydrolysis rates and aglycone patterns. These results confirm the incomplete adsorption of $C_{19}O_2$ and polar hydroxylated steroid diconjugates on the XAD-2 resin.

In order to improve the recovery of the diconjugates, the XAD-2 resin was washed with 200 ml of distilled water, followed by elution with 400 ml of methanol. Thus, the diconjugates with relatively poor recoveries ranging from 64 to 71% with the standard procedure were recovered to the extent of 75–85% using this modified procedure. For comparison, ^{3}H-TS and ^{3}H-TGA were treated by this procedure and these monoconjugates provided 99% recoveries. Additional studies were then made with sodium chloride and urea in order to determine to what extent inorganic and organic compounds were eluted in the methanol fraction. Each 100 mg of sodium chloride and urea was treated as above. Determination of these compounds revealed that they were recovered quantitatively in the aqueous fraction. Thus, the reduction in volume of water from 400 to 200 ml should have little influence on the contaminations by inorganic and organic compounds. High recoveries of the diconjugates were obtained on washing the XAD-2 resin with 100 ml of distilled water, followed by elution with 400 ml of methanol. However, 4% of the sodium chloride and 23% of the urea appeared in the methanol fraction. These results demonstrate that the reduction in volume of water results in a convenient procedure for recovering the diconjugates from the XAD-2 resin, although some contamination by inorganic and organic compounds is inevitable. However, the modified procedure should be especially applicable to the extraction of steroid diconjugates from sodium chloride solutions following Sephadex LH-20 or DEAE-Sephadex column chromtography of steroid conjugates, which employs 0.01 M sodium chloride as solvent or a 0–0.8 M sodium chloride gradient as the moving solvent phase.